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Biodegradation of P-Cresol by Mixed Culture in Batch Reactor – Effect of the Three Nitrogen Sources Used

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Abstract

Phenols are distributed either as nature or artificial mono-aromatic compounds in various environmental sites as major pollutants. Their existence in wastes from industrial processes such as oil refineries, coking plants, wastewater treatment plants, petroleum-based processing, phenols resin industry manufacturing and plants, has been well established. Many processes have been used for the remediation of Phenolic compounds. Conventional methods of treatment have been largely chemical or physical, but these processes have led to secondary effluent problems and costly. Biological treatment is an effective method that is used where many micro-organisms can be growing on Phenolic compounds as the sole source of carbon.

The temperature (30°C), the stirring velocity (200 r/min), NaH_2PO_4 concentration (3 g. L^{-1}), $MgSO_4$ concentration (0.1 g. L^{-1}), initial concentration of p-cersol (100 mg. L^{-1}) and KH_2PO_4 concentration (3 g. L^{-1}) for $(NH_4)_2SO_4$, (3 g. L^{-1}) for NH_4NO_3, (3 g. L^{-1}) for NH_4Cl; while the initial concentration of nitrogen sources ($(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl) was varied in the following range, 0 – 2 g. L^{-1}. All experiments were carried out at a given initial bacterial concentration, 0.08 g.L-1 (based on optical density determination, 0.079).

Irrespective of the culture conditions, total p-cresol degradation (100 m g. L^{-1}) was recorded for culture time ranging from 32.5 to 49 h. the optimal concentration were therefore, 1 g. L^{-1} for all nitrogen sources, leading to a specific growth rate of 0.3 h-1. Higher maximum specific growth rate values were recorded during this work, if compared to those reported in the available literature, even those dealing with mixed culture. This result showed the relevance of the specific microbial consortium used.

Two mathematical models were used to describe the length of lag phase versus initial nitrogen concentration; the growth length of lag phase was fitted using polynomial models, the correlation coefficient was varied in the following range, 0.99 – 1.

Keywords: *Biodegradation, P-cresol, Microbial consortium, Batch kinetics.*

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1. Introduction

A large number of organic compounds are discharged into waste stream of various industries. Among them, the cresols are highly toxic compounds. p-cresol is an isomeric phenol with a methyl substituent at the para position relative to hydroxyl group. p-cresol has a wide variety of uses including as disinfectants, fumigants, explosives, in the manufacturing of synthetic resins, in photographic developers, etc. It is a naturally occurring metabolic product that is formed from tyrosine by bacteria under anaerobic conditions [1]. In addition to being highly toxic and potential carcinogen, p-cresol causes, even at very low concentration, adverse effects on the central nervous system, cardiovascular system, lungs, kidney, and liver resulting in CNS depression [2]. EPA has classified p-cresol as a pollutant of group C (possible human carcinogens) [3]. The Ministry of Environment and Forest (MOEF), Govt. of India has set a maximum concentration level of 1.0 mg/L of p-cresol in the industrial effluents for the safe discharge into surface waters. The WHO recommends the permissible p-cresol concentration of 0.001 mg/L in potable waters [4]. Therefore to save our aqueous ecosystem, it is essential to reduce p-cresol concentration in wastewater to acceptable levels before safe disposal to water bodies.

Several conventional physiochemical techniques are available in the literature to treat wastewater. These include chlorination, ozonation, adsorption, membrane process, solvent extraction, flocculation, coagulation, etc. [5]. Most of them suffer from serious drawbacks such as high cost, tendency of the formation of secondary hazardous byproducts, incompleteness of purification. In recent years, wastewater effluents are being treated biologically due to environmental friendly nature of biotechnology.Biodegradation is the most cost effective method for complete destruction of organic pollutants [6]. The microorganisms play a vital role in biodegradation of toxic chemicals in the environment. In spite of the toxic properties of cresols, a number of microorganisms utilize cresol as sole source of carbon and energy required for their survival and growth, under aerobic operating conditions, even at relatively high concentrations [7].These microorganisms may be bacteria, yeast, algae or fungi and are capable to utilize phenol and its derivatives which are found in soil and water environments. The biodegradation of phenolic wastes has been extensively studied with various types of microorganisms [8–33]. After reviewing the literature on the degradation of p-cresol, it was found that most of the studies were carried out using bacteria [10,15,19,28,30], some using yeast [11,13] and algae [16,34]. Some microbial fungi have been recognized for their potential for phenol biodegradation, but the studies regarding the capabilities of fungi to degrade p-cresol in wastewater are insufficient [8,9].

Fungi are free living microorganisms, and generally live in soil and region of lower relative humidity. They are wide spread in nature and are considered primary decomposer in the world as they are responsible for oxidation of dead organic materials [35].

The main goal of this paper was to investigate the biodegradation of p-cresol by microbial consortium. In this aim, the nitrogen concentration was optimized.

The novelty of this work can be detailed in two aspects. Firstly, effect of the three nitrogen sources used to biodegrade p-cresol. Secondly, the modelization of length of lag phase for three nitrogen sources.

2. Materials and Methods

2.1 Microorganisms cultivation

The microbial consortium used in this work was obtained from the activated sludge of Boumerdès station (Algeria). Stock cultures were stored at + 4 °C. The microorganisms were activated for 24 h at 30 °C in a nutrient medium containing (g. L^{-1}): peptone, 15, yeast extract, 3, sodium chloride, 6, and (D+)-glucose, 1.

After 24 h, when cells were grown, the biomass was harvested by centrifugation. The microorganisms collected after centrifugation (3000 rpm for 30 min) were suspended in NaCl 0.5 % and re-centrifuged. After the third washing, the microorganisms collected after centrifugation were re-suspended again in NaCl 0.5 % and the microbial concentration was deduced from turbidimetric measurements. In this aim, after OD measurement at 600 nm (Vis spectrophotometer – HACH DR 2800), the OD value was converted to dry cell mass using a dry weight calibration curve. The dry cell mass density (g. L^{-1}) was found to follow the following regression equation x (g L^{-1}) = 1.044 × OD (600 nm).

Specific growth rate was determined in the exponential growth phase [36 - 39]. For each flask, it was determined from the time-course of the linear semi logarithmic plot of cell concentration during the exponential growth phase, namely when the specific growth rate was approximately constant [40].

2.2 Biodegradation experiments

For an OD value of the adapted cells in the range 2.7 – 2.9, an aliquot of the culture was centrifuged at 3000 rpm for 30 min. To wash the biomass, it was re-suspended in NaCl 0.5% and centrifuged. The cells (1 ml) were then transferred and inoculated in Erlenmeyer flasks (250 mL) to yield an initial OD of 0.078, and containing 100 mL of culture medium containing nitrogen source and the following mineral salt supplementation (MSS), namely NaH_2PO_4, KH_2PO_4 and $MgSO_4$ at the required concentrations, and 100 mg. L^{-1} of p-cresol. The cells were cultivated at 30 °C and 200 rpm. Samples were withdrawn at suitable time intervals and the biomass concentration was indirectly monitored by means of turbidimetric (OD) measurements as described above; while p-cresol was estimated using a U.V spectrophotometer at 298 nm.

3. Results and discussion

3. 1 Optimal nitrogen source supplementation

Fig. 1 - 3 show the effect of nitrogen concentration on p-cresol degradation. It should be noted that the optimal mineral salt supplementation was considered for these batch cultures as well as a neutral pH (7). Irrespective of the culture conditions, p-cresol biodegradation (100 mg. L^{-1}) was recorded within 48 h; the shortest time for p-cresol biodegradation was 32.32 h for $(NH_4)_2SO_4$ and NH_4Cl and 35.07 h for NH_4NO_3. The optimal concentration of nitrogen sources is same equal to 1 g. L^{-1}.

The evolution of cell concentration shows that the nitrogen source concentration has no noticeable effect on the biomass on substrate yield (not shown) and that the short lag time is equal to 24 h for $(NH_4)_2SO_4$ and NH_4Cl and 29.9 h for NH_4NO_3. Fig 1 -3 (b). show the effect of the nitrogen source concentration on maximum specific growth rate; its maximum value was 0.435 h^{-1} for $(NH_4)_2SO_4$, 0.347 h^{-1} for NH_4NO_3, 0.36 h^{-1} for NH_4Cl at same nitrogen concentration equal to 1 g L^{-1}.

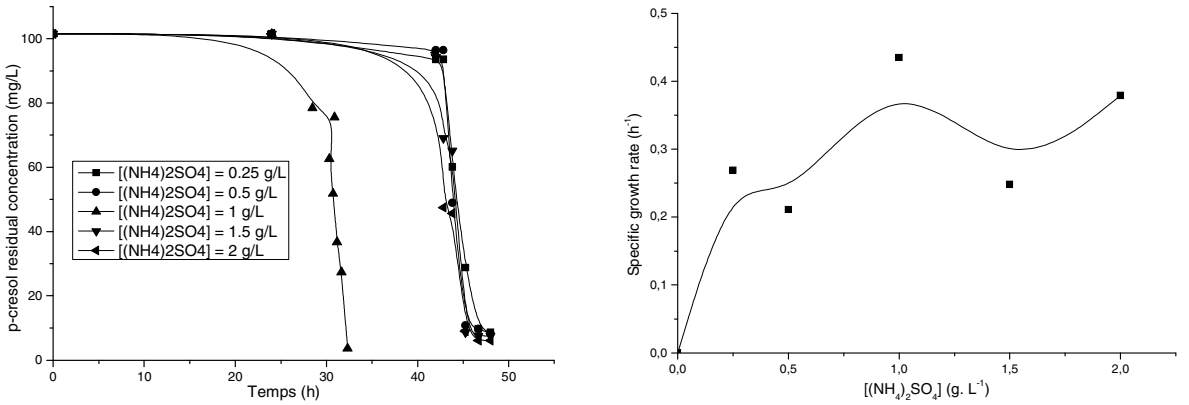

Fig.1. (a) Time-courses of the residual p-cresol concentration for different initial $(NH_4)_2SO_4$ concentration, (b) specific growth rate versus initial $(NH_4)_2SO_4$ concentration ($[NaH_2PO_4]$ = 3 g L^{-1}; $[KH_2PO_4]$ = 3 g L^{-1}; $[MgSO_4]$ = 0.1 g. L^{-1}; [p-cresol] = 100 mg L^{-1}; Temperature = 30 °C; stirring velocity = 200 r/min; pH = 7).

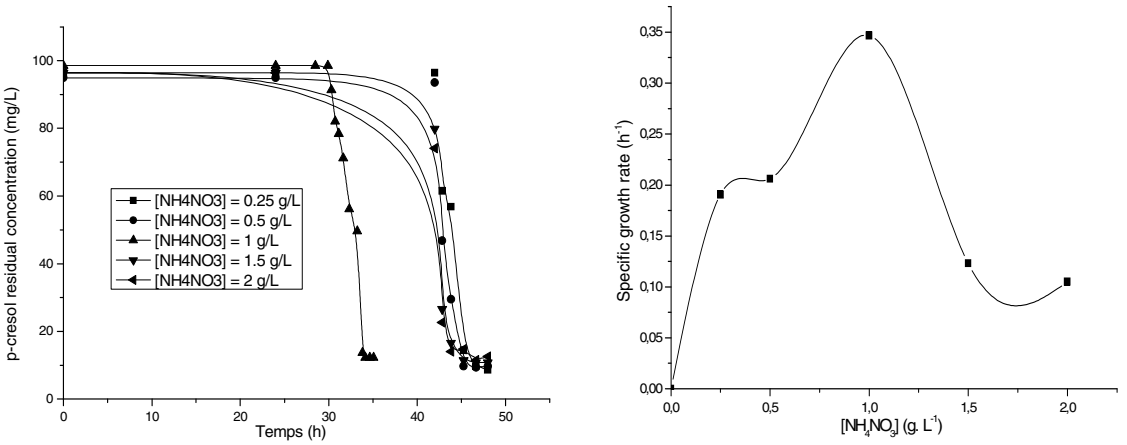

Fig.2. (a) Time-courses of the residual p-cresol concentration for different initial NH_4NO_3 concentration, (b) specific growth rate versus initial NH_4NO_3 concentration ([NaH_2PO_4] = 3 g L^{-1}; [KH_2PO_4] = 1 g L^{-1}; [$MgSO_4$] = 0.1 g. L^{-1}; [p-cresol] = 100 mg L^{-1}; Temperature = 30 °C; stirring velocity = 200 r/min; pH = 7).

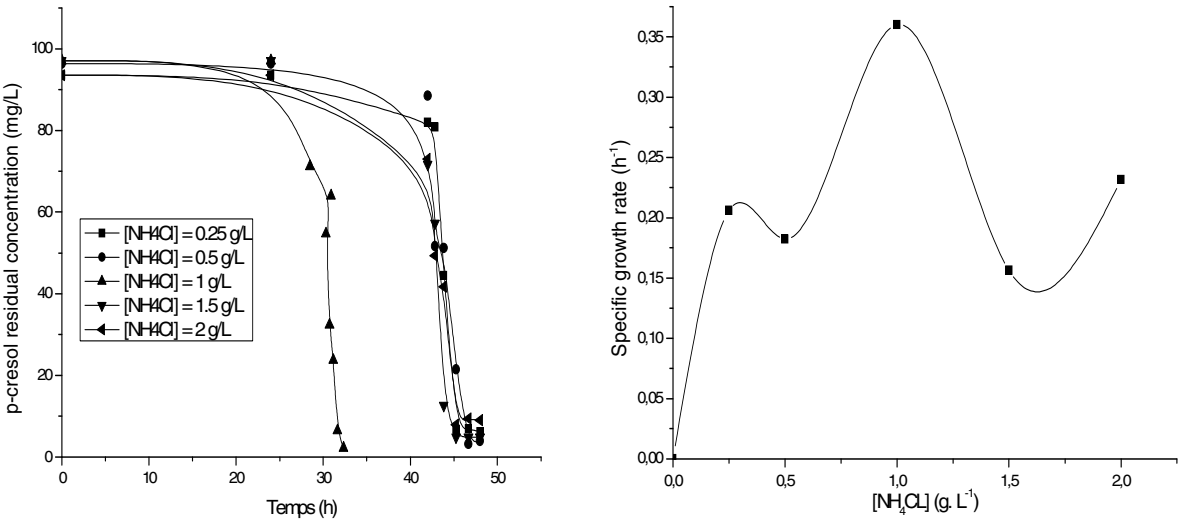

Fig.3. (a) Time-courses of the residual p-cresol concentration for different initial NH_4Cl concentration, (b) specific growth rate versus initial NH_4Cl concentration ([NaH_2PO_4] = 3 g L^{-1}; [KH_2PO_4] = 4 g L^{-1}; [$MgSO_4$] = 0.1 g. L^{-1}; [p-cresol] = 100 mg L^{-1}; Temperature = 30 °C; stirring velocity = 200 r/min; pH = 7).

3.2 Length of lag phase t0

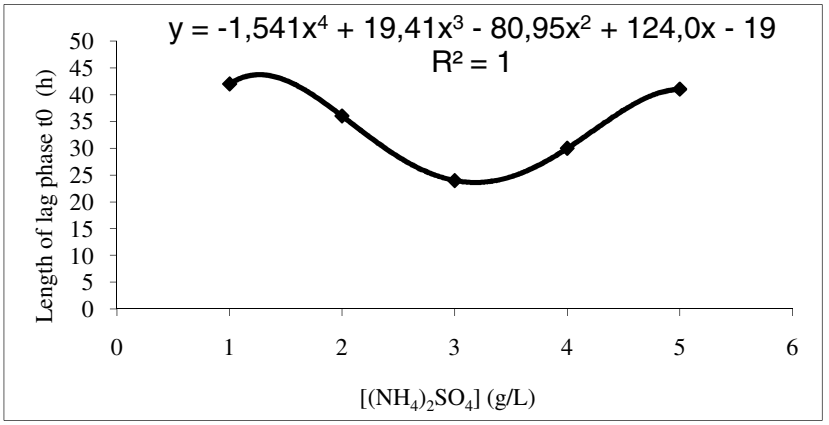

Fig. 4. Length of lag phase t0 versus initial $(NH_4)_2SO_4$ concentration

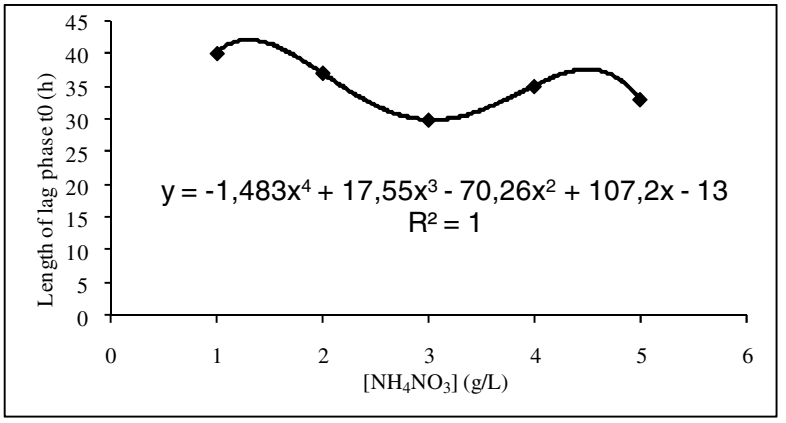

Fig. 5. Length of lag phase t0 as function of initial NH_4NO_3 concentration

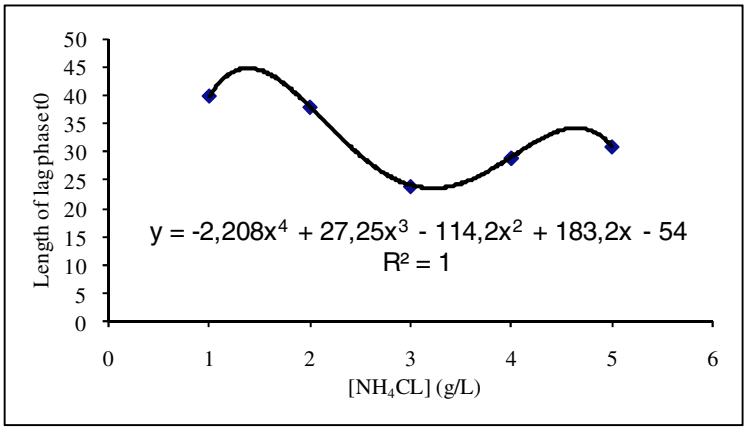

Fig. 6. Length of lag phase t0 as function of initial NH_4Cl concentration

Fig. 4 - 6. show polynomial evolution Length of lag phase t0 versus initial nitrogen concentration where polynomial degrees is equal to 4 for all nitrogen sources. The correlation coefficient is equal to 1.

4. Conclusion

The degradation of p-cresol by three nitrogen sources were studied., p-cresol biodegradation (100 mg. L^{-1}) was recorded within 48 h; the shortest time for p-cresol biodegradation was 32.32 h for $(NH_4)_2SO_4$ and NH_4Cl and 35.07 h for NH_4NO_3. The optimal concentration of nitrogen sources is same equal to 1 g. L^{-1}. The maximum value of specific growth rate was obtain (0.435 h^{-1} for $(NH_4)_2SO_4$, 0.347 h^{-1} for NH_4NO_3, 0.36 h^{-1} for NH_4Cl) when nitrogen concentration equal to 1 g L^{-1}. The short lag time is equal to 24 h for $(NH_4)_2SO_4$ and NH_4Cl and 29.9 h for NH_4NO_3.

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